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TITLE: Developing an Angiogenic Therapy to Enhance and Accelerate Repair of Traumatic Bone Defects

PRINCIPAL INVESTIGATOR: Steven R. Buchman

CONTRACTING ORGANIZATION: University of Michigan, Ann Arbor, MI

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14. ABSTRACT

Our technology works through the stimulation of angiogenesis, an early and critical component of bone repair that subsequently allows for the recruitment of endogenous cell populations and for the optimization of the soft tissue envelope required to enhance bone healing and regeneration. The overarching goal of this proposal is to expedite the healing of traumatic bone defects incurred on the battlefield. We are proposing the advancement of an implantable microparticle technology that will function to accelerate fracture healing and fill large segmental bone defects.

15. SUBJECT TERMS
None listed.

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1. Introduction

Full thickness segmental bone defects secondary to blast injuries represent perhaps the most devastating skeletal wounds incurred on the battlefield. These complex injuries are difficult to reconstruct owing to the need to replace or regenerate missing bone in the setting of severely impaired vascularity and injured surrounding tissues. In pre-clinical animal studies, the full thickness segmental defect is modeled by the critical size defect (CSD), which represents a bone defect spanning a gap that will not heal normally without therapeutic intervention. Therefore, the purpose of this study is to investigate the efficacy of a therapeutically augmented vascular response to injury in order to significantly enhance the healing of long bone CSDs. Our goal is to optimize our hyaluronic acid-deferoxamine therapy (Ferroximend), generate a hydrogel formulation for ease of clinical administration, and demonstrate its efficacy in healing our femoral CSD rat model.

2. Keywords

Critical size defect, bone, femur, hyaluronic acid, deferoxamine, Ferroximend, vascularity, regeneration

3. Accomplishments

- **What were the major goals of the project?**
    - Establish baseline CSD metrics
        - *Milestone target: 18 months (February 2024)*
        - *Current completion: Approximately 60% finished; target date still achievable*
    - Employ Ferroximend Treatment
        - *Milestone target: 35 months (August 2025)*
        - *Current completion: Not initiated; target date still achievable*
- **What was accomplished under these goals?**
    - During the previous reporting period, our collaborators at Hylapharm developed 16 formulations of Ferroximend for us to run in vitro tubule formation assays in order to determine the best candidates for the final hydrogel therapy. We were able to differentiate several promising formulations from the batch of 16 and have returned our results to Hylapharm. We expect to have an animal-ready hydrogel product from Hylapharm early next year that takes into account therapeutic efficacy as well as ease-of-delivery.
    - Additionally, in our animal model, we performed a small pilot study with 2mm, 3mm, and 5mm femoral defects to determine which would be most appropriate for this study. 3mm proved to be a critical size defect, and therefore we are employing that in our model moving forward. Our control groups have undergone the surgeries and are currently in the 10-week healing period. We will perform all our outcomes metrics upon sacrifice of the animals.
- **What opportunities for training and professional development has the project provided?**

    Nothing to report.

- **How were the results disseminated to communities of interest?**

  Nothing to report.

- **What do you plan to do during the next reporting period to accomplish the goals?**

  - During the next reporting period, we plan to finish the baseline CSD metrics, including the mineralization, vascularity, mechanical testing, and histologic parameters described in our proposal. Additionally, we should have a hydrogel formulation of Ferroximend early in 2024, at which point we can begin our treatment studies in our animal model.

4. Impact

Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

- **What was the impact on the development of the principal discipline(s) of the project?**

  Nothing to report.

- **What was the impact on other disciplines?**

  Nothing to report.

- **What was the impact on technology transfer?**

  Nothing to report.

- **What was the impact on society beyond science and technology?**

  Nothing to report.

5. Changes/Problems

- **Changes in approach and reasons for change**

  - Nothing to report.

- **Actual or anticipated problems or delays and actions or plans to resolve them**

  - Our only delay was in obtaining ACURO approval for our animal studies. We initially submitted our request for approval on August 22, 2022 and then were asked by ACURO to revise our animal protocol on September 1, 2022. We made the changes, our university IACUC approved the changes, and we submitted them to ACURO on September 28, 2022. We followed up monthly, but did not receive a reply until December 21, 2022, at which point our DOD contact told us that her email had changed a few months prior. We received final ACURO approval on January 12, 2023. As such, the initiation of our animal work was delayed 4 months, but we have run studies in parallel rather in sequence in order to make up for lost time. We believe we are back on track to finish our milestones on time.

- **Changes that had a significant impact on expenditures**

  - The delay prevented us from ordering animals and surgical supplies during the first four months of the project, and therefore our first-year expenses are lower than previously budgeted. The expenses should be higher in subsequent years as a result.

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

  - No significant changes to report.

6. Products

- We have no finalized products to report.

7. Participants & Other Collaborating Organizations

What individuals have worked on the project?

Name:	Steven Buchman
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0001-9953-0834
Nearest person month worked:	Less than 1
Contribution to Project:	Dr. Buchman has oversight on this project and all animal work and in vitro testing is performed in his laboratory.
Funding Support:	

Name:	Alexis Donneys
Project Role:	Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):	0000-0001-8690-0971
Nearest person month worked:	2
Contribution to Project:	Dr. Donneys has led the animal surgeries associated with this project and helped with animal husbandry and day-to-day tasks.
Funding Support:	

Name:	Noah Nelson
Project Role:	Lab Technician
Researcher Identifier (e.g. ORCID ID):	0000-0002-2972-442X
Nearest person month worked:	9
Contribution to Project:	Mr. Nelson has performed the in vitro assays on the Ferroximend candidates and assisted with all animal work.
Funding Support:	

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

- Nothing to report.

What other organizations were involved as partners?

i. **Organization Name:** Hylapharm, LLC

ii. **Location of Organization:** Lawrence, KS

iii. **Partner's contribution to the projec**t

Other: Hylapharm produces the HA-DFO (Ferroximend) product to our specifications. They produced the 16 preliminary prototypes used in the first stages of the experiment and will take our feedback and create the optimal hydrogels for the second half of the project.

8. Special Reporting Requirements

Not applicable.

9. Appendices

Not applicable.